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MRID No. 444577-32

DATA EVALUATION RECORD
§ 72-3 - ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE FISH

1. **CHEMICAL:** Prohexadione Calcium — PC Code No.: 112600

2. **TEST MATERIAL:** BAS 125 W — Purity: 90.6%

3. **CITATION:**

Authors:	W.C. Graves, J.P. Swigert, and C.M. Holmes
Title:	BAS 125 W: A 96-Hour Static-Renewal Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*)
Study Completion Date:	April 14, 1997
Laboratory:	Wildlife International Ltd., Easton, MD
Sponsor:	BASF Corporation, Agricultural Products, Research Triangle Park, NC
Laboratory Report ID:	147A-146
MRID No.:	444577-32
DP Barcode:	D245631

4. **REVIEWED BY:** Karl Bullock, M.S., Environmental Scientist, Golder Associates Inc.

Signature: Karl Bullock **Date:** 7/7/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

Signature: P. Kosalwat **Date:** 7/7/98

5. **APPROVED BY:**

Signature: [illegible] **Date:** 11/17/97

6. **STUDY PARAMETERS:**

Age or Size of Test Organism:	18-24 mm
Definitive Test Duration:	96 hours
Study Method:	Static-Renewal
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using an estuarine fish. Based on mean measured concentrations, the 96-hour LC_{50} was determined to be >122 ppm ai, which classifies BAS 352 F as practically non-toxic to the sheepshead minnow. The NOEC was determined to be 122 ppm ai.

Results Synopsis

LC_{50}: >122 ppm ai 95% C.I.: N/A

NOEC: 122 ppm ai Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. Acclimation period (52 hrs) was shorter than recommended (14 days).

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the sheepshead minnow (*Cyprinodon variegatus*) or the silverside (*Menidia* spp.).	*Cyprinodon variegatus*
Mean Weight 0.1-5 g	Mean: 0.29 g Range: 0.17 - 0.47 g
Mean Standard Length Longest not > 2x shortest	Mean: 20 mm Range: 18 - 24 mm
Supplier	In-house cultures
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	52 hours
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	Not fed 68 hours prior to or during testing.
Pretest Mortality < 3% mortality 48 hours prior to testing	Not reported.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Reconstituted seawater or seawater from a natural source.	Natural seawater pumped from Indian River Inlet, DE, passed through a sand filter, aerated, and diluted to salinity of approximately 20 ‰ with Wildlife International Ltd. well water.
Does water support test animals without observable signs of stress?	Yes
Salinity Weekly range should not deviate by more than 6%.	20 ‰
Water Temperature 22°C	21.4 - 23.0°C

Guideline Criteria	Reported Information
pH Monthly range must not deviate by more than 0.8 unit. Euryhaline: 7.7-8.0 Stenohaline: 8.0-8.3	8.0 - 8.3
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	≥65% throughout test
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	 Glass 18.9 L 15 L
Type of Dilution System Must provide reproducible supply of toxicant	N/A
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	Test solutions were renewed on Day 2
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.19 g/L/day
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	None

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	A range-finding test with negative control and nominal concentrations of 0.97, 3.2, 11, 36, and 120 mg ai/L resulted in 0% mortality and no sublethal effects at all concentrations.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Negative control and nominal concentrations: 16, 26, 43, 72, and 120 mg ai/L.
Number of Test Organisms Minimum 10/level for static test, 20/level for flow-through, may be divided among containers	10 per replicate, 20 per treatment level
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Temperature and DO were measured at test initiation and termination as well as prior to and after each renewal (old and new solutions) in all aquaria and continuously in one negative control replicate. pH was measured at test initiation, prior to and after the renewal, and at test termination in alternate replicate test chambers.

Guideline Criteria	Reported Information
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Yes, solutions were collected from each test chamber at test initiation and termination, as well as at 48 hours from replicate A of the "old" solutions and replicate B of the "new" solutions and analyzed by HPLC.

12. **REPORTED RESULTS:**

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	Average: 94-102% of nominal
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	No signs of test material toxicity were observed

Mortality

Concentration (mg ai/L)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	<0.05	20	0	0	0	0
16	15	20	0	0	0	0
26	26	20	0	0	0	0
43	44	20	0	0	0	0
72	72	20	0	0	0	0
120	122	20	0	0	0	0

Other Significant Results: No signs of test material toxicity were observed.

B. Statistical Results

Method: Visual inspection

96-hr LC_{50}: >122 mg ai/L 95% C.I.: N/A

Probit Slope: N/A NOEC: 122 mg ai/L

13. **VERIFICATION OF STATISTICAL RESULTS:**

Method: Visual inspection

96-hr LC_{50}: >122 ppm ai 95% C.I.: N/A

Probit Slope: N/A NOEC: 122 ppm ai

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using an estuarine fish. Based on mean measured concentrations, the 96-hour LC_{50} of >122 ppm ai, classifies BAS 125 W as practically non-toxic to the sheepshead minnow. The NOEC was determined to be 122 ppm ai. This study is classified as **Core**.